

The University of Chicago

ON
THE EXISTENCE OF PRO-SECRETIN

A PART OF A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY
1934

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ON THE EXISTENCE OF PRO-SECRETIN

In 1902 Bayliss and Starling published their original researches on the hormone control of the external secretion of the pancreas. They found that acid solutions would extract secretin from the mucosa of dog duodena while neutral solutions would not affect the extraction, at least not nearly so well. For that reason they postulated the existence of a precursor, prosecretin, which was hydrolyzed by acids to yield active secretin.

Since that time there have been many investigations on the secretin problem. In the main, however, they have been directed toward the proof of the physiological part played by secretin and toward the isolation of the hormone. A much smaller number have been concerned with the question of the existence of prosecretin. For that reason many of the published opinions concerning the prohormone have been made from incidental observations, rather than from directed experiments planned to give information concerning its existence or properties. It is only in recent years that the proof of the rôle of secretin in the digestive process has been accepted by the majority of the workers in the field, and it is therefore not surprising that this particular problem has been relatively neglected.

The literature on the subject has been recently reviewed by Still (1931) and although a bibliography is appended to this paper, a review of the literature will not be included here.

In this study we have reopened the question and have attempted to obtain concrete evidence concerning the existence of prosecretin.

EXPERIMENTAL. In order that the work in all the experiments reported in this paper would be comparable, all were of one general plan. Except in special instances, all extracts of the mucosa were made in the ratio 1:20 with respect to the particular solvent used. The insoluble residues were removed by centrifugation, the extracts were neutralized and 5 cc. portions were injected intravenously into dogs prepared for secretin assay in the manner described in the review by Still (1931). In any given experiment, then, a fairly accurate comparison of the activity of the various extracts was obtained and a less accurate comparison of the activity of extracts used in different experiments. A description of the methods

used in preparing the extracts and special points concerning some of the experiments, will be included in the proper place in the text of the paper.

The experiments were planned with the hope of preparing, and possibly purifying, an extract of the duodenal mucosa which would possess certain properties, namely, that the intravenous injection of it would cause no secretion of pancreatic juice until acidified or that the secretagogue potency already present would be greatly increased by acidification.

Series I. Experiments on "untreated mucosa." In these experiments, fresh mucosa was thoroughly ground with sand and extracted with the solvent used. The mixture was centrifuged and the filtrate neutralized, when necessary. The neutralized filtrate was assayed in 5 cc. quantities.

Dog mucosa. A. Fresh dog mucosa was extracted with 0.9 per cent sodium chloride solution, in the manner described above and the extract was divided into two portions "a" and "b." Extract "a" was injected without change or additions to it. Extract "b" was strongly acidified with HCl and after standing for a few minutes an equivalent amount was

TABLE 1

The effect of acidification of neutral saline extracts of fresh duodenal mucosa of the dog

EXTRACT	TREATMENT OF THE MUCOSA	PANCREATIC RESPONSE
		cc.
"a"	Neutral saline extract	0.3
"b"	Neutral extract—acidified	0.2
"c"	Acid extract of the residue	2.2

neutralized and injected for assay. The residue, from the above process, was extracted with 0.4 per cent HCl and centrifuged; the filtrate "c" was neutralized and injected for assay. Table 1 shows the results of one typical experiment.

By reextracting fresh dog mucosa with neutral saline from four to ten times, the quantity of secretin which could be extracted with saline in succeeding extracts rapidly diminished to sub-threshold quantities. The residue, however, upon acid extraction yielded abundant secretin.

When the cellular continuity of the mucosa was destroyed by repeated freezing with CO₂ snow and ether, the results were similar to those already noted except in a few instances in which the saline extract "b" developed slightly more activity upon acidification than was found in the saline extract "a." This can be considered only as mildly suggestive evidence for the existence of prosecretin because the increase was small and such preparations all contained large amounts of noncentrifugable material. Furthermore, the extracts made from the "frozen" mucosa were extremely toxic and it was felt that data from them should be used only as confirmatory evidence.

Similar experiments were carried out in which "frozen" mucosa was dropped into boiling 0.4 per cent HCl or 0.9 per cent NaCl solutions, then cooled and centrifuged. In this way the coagulable protein was removed. The secretin content of these filtrates was found to be in the approximate ratio of 20:1.

Hog mucosa. B. Experiments similar to those described under "A" were conducted, using fresh hog mucosa. The results were essentially the same as the above experiments employing fresh dog mucosa, except for one feature. The amount of saline extractable secretin was more in every instance than that found in the case of dog mucosa. In fact, it was found frequently that almost as much saline extractable as acid extractable secretin was present in the fresh hog mucosa. This difference is not due to differences in freshness of the mucosa and may be due to a species difference.

These results are similar to those of Bayliss and Starling since they show that prosecretin is not soluble in neutral saline. In addition, these experiments show the following: 1. A portion of the secretin is present in the mucosa in an "active" form and may be extracted with neutral saline. The quantity of this saline extractable secretin is variable from animal to animal and there is apparently a greater difference in the relative amounts in different species of animals. 2. After repeated extractions of the "fresh untreated" mucosa with saline, further saline extracts of this mucosa do not contain a threshold quantity of secretin. The residue, however, will yield a considerable quantity of secretin to acid extraction.

It should also be mentioned that reagents other than acids may extract secretin from the fresh mucosa which has been repeatedly washed with saline as described above; such solutions as dilute alkali and aqueous alcohol belong to this group, the alcohol being much more efficient than the dilute alkali.

The preparations just described in this series have contained large quantities of vasodilatin, together with other toxic substances. Attempts were made to "salt-out," with neutral salts, a relatively non-toxic precipitate from slightly alkaline saline suspensions of fresh mucosa. It was hoped that the precipitate would contain not only secretin but its precursor, prosecretin. These experiments were unsuccessful in the latter respect because all of the secretin present in the precipitate could be removed by neutral saline and acidification of the saline extract did not enhance its secretagogue action. Experiments along this line were discontinued.

Series II. Experiments on dried mucosa. The question was raised during the progress of the experiments of series I, concerning the ability of saline to penetrate the cells of the mucosa rapidly enough to extract the secretin. The following experiments were planned with that point in mind. It should be mentioned that Stepp (1912) was probably the first to work

with dried mucosa. His product contained secretin which could be extracted by saline, acids, bile, glycerine and aqueous alcohol. This finding led him to suggest several kinds of secretin.

A. *The "dried mucosa."* Preparations were made by the following procedure. Fresh mucosa of the dog was spread in a thin layer on glass plates and dried in a current of warm air. The dried scales were then milled to pass through a 400 mesh wire sieve.

This preparation yielded only a small amount of secretin on extraction with saline, dilute alkali, and bile salts. However, aqueous alcohol and acids extracted considerable secretin from the dried preparation, the latter extractant being the most efficient. The relative amounts of secretin extracted from the mucosa which had been dried, by these solvents, was of the order obtained from the "untreated fresh mucosa" (series I) treated with the same solvents. It was discovered that the preparation developed considerable acidity during the drying process. The need of a dried preparation which remained neutral or slightly alkaline, was obvious.

B. *"Dried-heated-mucosa."* In order to eliminate as much as possible the development of acidity, the preparation was made in a manner similar to the above (A. "Dried mucosa") except for a preliminary heat treatment of 30 minutes' duration at 80°C., to reduce the effects of enzymes and bacteria.

This product was similar to A ("dried-mucosa") except that more secretin, which was soluble in saline, was present in this mucosa.

C. *"Dried-heated-alkaline-mucosa."* To further eliminate the possible effects of acid development, preparations were made which were kept slightly alkaline (to phenolphthalein) but otherwise prepared as the "heated-dried-mucosa" (B).

This preparation reacted to solvents in a manner similar to the above mentioned preparation.

It should be noted with respect to these preparations that heating causes more of the secretin to become saline soluble. Table 2 shows the results of an experiment in which aliquot samples of the same mucosa were used in making of "dried-mucosa" (A), and "dried-heated-mucosa" (B), of both hog and dog mucosa. The table also indicates the larger amount of saline soluble secretin in the saline extracts.

D. *"Alkaline-crude."* If one extracts the "dried-heated-alkaline-mucosa" (C) repeatedly with saline, the saline soluble secretin can be removed. The residue may be dried with acetone and ether and preserved as a dry powder which possesses the following properties: a. The preparation will yield secretin activity to: 1, dilute mineral acids; 2, stronger organic acids; 3, seventy per cent aqueous alcohol; 4, hot neutral saline. b. The preparation will not yield secretin activity to: 1, NaCl solutions up to 5 per cent; 2, NaOH solutions up to 0.5 per cent; 3, sodium carbonate, 0.2 per cent; 4, solutions of bile salts.

Discussion of series I and II. These experiments show conclusively that the secretin activity of fresh mucosa is not all in the same state, because it is possible, using fresh mucosa (series I) or treated mucosa (series II), to wash out the saline soluble fraction so that further saline extraction fails to yield any activity. However, subsequent extraction of the residue with acids yields a considerable amount of secretin.

Although the experiments in series II are subject to the criticism that proteins of the mucosa have been denatured and possibly otherwise altered by reagents, the experiments of series I are not subject to that criticism.

These results might be interpreted in several ways, among which are: 1. The saline insoluble fraction represents prosecretin. 2. The saline insoluble fraction represents active secretin adsorbed on some of the colloids. 3. The saline soluble fraction represents prosecretin which has been activated by digestive acids but has not been absorbed into the blood stream.

Since we were unable to test the first interpretation and were able to examine the second and third, experiments were planned to that end.

TABLE 2

PREPARATION	ANIMAL	TREATMENT OF MUCOSA	PANCREATIC RESPONSE
			CC.
A	Dog	Unheated	0.2
B	Dog	Heated	1.5
A	Hog	Unheated	1.6
B	Hog	Heated	2.1

Series III. The adsorption of secretin. Recognizing the fact that the product "alkaline crude" (D) may have been denatured in the making, it was, however, selected for these experiments because it contained no saline soluble secretin and further saline extraction would not materially alter its composition. We have assumed the latter to be also the case of fresh, washed mucosa.

Two kinds of experiments were carried out to test the possibility of adsorption.

The secretin in the "alkaline crude" mucosa was inactivated with trypsin. The trypsin was destroyed by heating and the residue tested to show the absence of acid soluble secretin. Purified secretin was then added and the pH adjusted to approximately eight. After stirring for fifteen minutes the solid material was separated by centrifugation and the residue washed repeatedly with neutral saline. The residue was then dried with acetone and ether. The results showed that the powder yielded no secretin to extraction with saline but considerable secretin to acid extraction.

Eight experiments were performed in the following manner: One gram

of the "alkaline crude" preparation was extracted with 20 cc. of 0.4 per cent HCl and the filtrate divided into two portions, "a" and "b." Extract "a" was neutralized and 5 cc. injected intravenously for assay; extract "b" was made slightly alkaline (to phenolphthalein) and allowed to stand at room temperature for 15 minutes before injection. These extracts were prepared to demonstrate the quantity of secretin which the preparation would yield to acid extraction and to determine the effect of weakly alkalinizing such a filtrate. One gram of the "alkaline crude" preparation was then extracted with 20 cc. of 0.4 per cent HCl, the pH of the mixture was adjusted to about eight with NaOH and then stirred for 15 minutes. The mixture was then centrifuged and 5 cc. of the filtrate (just alkaline) constituted extract "c." Extract "d" was a 0.4 per cent HCl extract of the residue from "c." Extract "c" was prepared to determine if the adjustment of the pH results in adsorption of secretin. "d" was prepared to determine if the secretin which was adsorbed in "c" and

TABLE 3
Experiment showing the adsorption of secretin on activity "free" mucosa

EXTRACT	NATURE OF EXTRACT	PANCREATIC RESPONSE
		cc.
"a"	Control on "alkaline-crude" preparation to acid extraction	3.2
"b"	Effect of alkali on acid filtrate from the "alkaline-crude" preparation	2.6
"c"	Adsorption of secretin on activity "free" mucosa	0.0
"d"	Acid extract from the residue of "c"	2.8

which was not extractable with neutral saline, could be eluted with an acid solution. Typical results are shown in table 3.

These experiments indicate that in "heated-dried-washed-mucosa" preparations, the residual secretin which is soluble in acids may be adsorbed on the mucosa. This does not prove that secretin is so held in the living mucosa but only indicates that the treated mucosa can adsorb secretin at the proper pH. We are aware of the fact that the previous history of the mucosa may influence considerably such processes.

Series IV. The effect of the exclusion of digestive acids on the state of secretin in the mucosa. Thiry-Vella fistulae were made in ten dogs. The fistulae included the lower duodenum and upper jejunum. The animals were kept for six months or longer during which time the fistulae were frequently flushed with neutral saline. Thus the fistulae did not come in contact with free acids other than those which were formed during the metabolism of the tissues. At the end of the period the animals were sacri-

ficed and the fistulae immediately removed. The mucosa was scraped off and aliquot portions used to prepare extracts for secretin assay. These extracts compared at that time with extracts similarly prepared from normal dog mucosa.

The results were quite typical of those obtained with other fresh mucosae. The solutions which were effective in extracting secretin were, in order of increasing efficiency: saline, dilute alkali, aqueous alcohol and acids. These experiments strongly indicate that the saline extractable fraction of secretin, obtained previously, is not present due to the effect of digestive acids.

SUMMARY

It has been shown that the secretin in "untreated mucosa" exists in two forms with respect to the ability of solutions to extract it, one fraction which is readily extractable with neutral saline, the other which is extractable with acids and is not extractable with saline.

The saline extractable fraction might be termed "free" secretin and the fraction not extractable with saline called "bound" secretin. The "bound" secretin may also be extracted to a lesser degree by weak bases and aqueous alcohol.

We are not in a position to say anything concerning the condition in which "bound" secretin is found in the mucosa. However, we believe the "acid-hydrolysis" hypothesis of Bayliss and Starling not to be entirely tenable, inasmuch as there are many reagents other than acids, such as dilute alkali, aqueous alcohol, etc., which will extract the secretin. Our results only indicate that the "bound" secretin may be held by adsorption to the colloids of the mucosa.

It is not at all improbable that the "bound" secretin is combined with or adsorbed on protein molecules. The reagents which effectively extract it do so by hydrolysis or elution. In that respect "pro-secretin" probably does exist.

Whether or not the "free" and liberated "bound" secretin are the same substance is undetermined.

REFERENCES

- BAYLISS, W. M. AND E. H. STARLING. *J. Physiol.* **28**: 325, 1902.
J. Physiol. **29**: 174, 1903.
BABKIN, B. P. *Die Aussere Sekretion der Verdauungsdrusen.* Berlin, 577, 1928.
CAMUS, M. L. *J. Physiol. et Pathol. Gen.* **4**: 1002, 1902.
DELEZENNE, C., AND E. POZERSKI. *Compt. rend. Soc. de Biol.*, **56**: 987, 1904.
J. Physiol. et Path. Gen. **14**: 540, 1912.
FLEIG, C. *J. Physiol. et Path. Gen.* **6**: 32, 1904.
FROVIN, A. AND S. LALOU. *Compt. rend. Soc. de Biol.* **71**: 189, 1911.

- GLEY, E. Compt. rend. Acad. d. Sci. **151**: 345, 1910.
J. Physiol. et Path. Gen. **14**: 509, 1912.
- MATSUO, I. J. Physiol. **45**: 447, 1912.
- MELLANBY, J. AND St. G. HUGGETT. J. Physiol. **61**: 122, 1926.
- STEPP, W. J. Physiol. **43**: 441, 1912.
- STEPP, W. AND E. SCHLAGINTWEIT. Ztschr. Biol. **62**: 202, 1913.
- STILL, E. U. Physiol. Rev. **11**: 328, 1931.
- VOLBORTH, G. W. This Journal **62**: 331, 1925.

